β-Amyloid Aggregation Inhibitors for the Treatment of Alzheimer's Disease: Dream or Reality?

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Abstract. Amyloid (Aβ) deposition remains a hallmark in the pathology of Alzheimer's disease (AD). Important drug discovery efforts dedicated to the inhibition of the polymerization process leading to amyloid neurotoxicity are pursued by academic groups and the pharmaceutical industry as a potential preventive treatment for AD. The aim of this review is to up-date current knowledge on the amyloid aggregation process and the various available peptidic and non-peptidic Aβ aggregation inhibitors.

Alzheimer's disease (AD), characterized by a progressive loss of memory as well as cognitive function, affects around 15 million people worldwide. The incidence increases from 0.5% per year at the age of 65 years to about 8% per year after the age of 85 years [1]. The prevalence of the pathology increases from 3% at the age of 65 years to 47% after the age of 85 years [2].

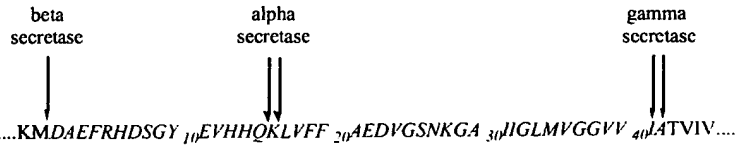
Current treatment focus on symptomatic aspects of the pathology and includes drugs increasing cholinergic neurotransmission, like the acetylcholine esterase inhibitors Tacrine, Donepezil or Rivastigmine [3, 4]. This therapy is devoid of any major impact on the progression of the disease and several preventive/curative approaches are currently developed. Some of these have already been tested in the clinic (e.g. combination of vitamin E and the monoamine oxidase inhibitor Selegiline); unfortunately without revealing any therapeutic benefit [5].

I. HOW DO WE HOPE TO TREAT ALZHEIMER'S DISEASE?

The goals for the treatment of patients suffering AD are to improve, or at least to slow, the loss of memory and cognitive function, without the induction of serious side effects. Moreover, the development of drugs that attack more fundamental processes of AD, preventing them from damaging function and quality of life, are eagerly awaited.

The growing understanding of the huge number of factors involved in AD leads to potential interesting avenues for drug discovery to modify disease progression, e.g.:

- *Anti-inflammatory agents*. One of the hallmarks of AD is inflammation in the brain. Epidemiological evidence strongly suggests that anti-inflammatory agents, like NSAIDs (Ibuprofen, Indomethacin) are associated with a reduced risk for AD [6, 7].
- *Antioxidants*. It has been recognized for a long time that over-production of free radicals results in oxidative stress that may play a role in AD. Many clinical trials using anti-oxidant agents have been performed, one of them involving Ginkgo biloba seems particularly promising [8].
- *Neurotrophic factors*. Neurotrophic factors (NGF, BDNF etc....) have demonstrated regenerative properties in various animal models and appear to represent a promising therapeutic avenue. A very interesting neurotrophic mimetic, AIT-082, a hypoxanthine derivative, is presently under clinical evaluation in AD [9].



Another hallmark of AD, the deposition of the β-amyloid (Aβ) peptide in the core of the senile plaques and in the walls of cerebral blood vessels [10], has induced a vast area of research focused on this 39-42 amino acid peptide:

II. THE ALZHEIMER AMYLOID β PEPTIDE

The physiological activity (if any) of Aβ, a normal constituent of biological fluids [11], is still unknown. It can

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exert neurotrophic [12] or neurotoxic effects [13-15] depending on its concentration and aggregation state. Indeed, it has been shown that only amyloid aggregates deriving from Aβ oligomers, having adopted a β-pleated sheet conformation [16, 17], are toxic to neurons. It is generally accepted that Aβ toxicity requires the assembly of Aβ aggregates into fibrils [18]. However, this view may be too simplistic and it appears probable that the fibrillogenesis process includes other (possible toxic) intermediates that might constitute the real drug discovery targets to be pursued.

Aβ seems to be involved in many neurotoxic pathways relevant for AD such as oxidative stress [19] and loss of calcium homeostasis [20, 21]. A dyshomeostasis regarding some metals found in the brain (e.g. zinc, copper) has also been reported in AD, and it should be noted that these "biometals" also promote Aβ aggregation in vitro [21, 22].

Aβ is formed from the cleavage of a larger species namely the β-amyloid precursor protein (βAPP), and, once released, is prone to self aggregation [23]. APP is processed via enzymes called secretases, amyloid being generated via the activity of the so-called beta and gamma secretases [24, 25], alpha-secretase producing non toxic APPs (soluble APP fragment). This area of research, with the recent discovery that γ secretase and presenilins 1 (PS1) and 2 (PS2) may be the same molecular entity, provides attractive targets for the lowering of amyloid production [26-28]. The identity of β-secretase has been established last year, with the discovery of the transmembrane aspartic protease BACE, Beta site APP-Cleaving Enzyme [29, 30].

Genetic evidence supporting the hypothesis that Aβ plays a key role in the pathogenesis of AD [31] is listed below:

- Three familial forms of AD (trisomy 21, βAPP gene mutations, and presenilin gene mutations) increase the production of Aβ.
- One of the most prominent genetic risk factor for AD (apolipoprotein E4) is associated with an increased Aβ deposition.
- Aggregated Aβ is toxic to neurons in vitro and in vivo.
- Transgenic mice overexpressing human mutated APP develop certain pathological features of AD like cerebral amyloid plaques, as well as cognitive dysfunction.

A. Amyloid Targeted Therapeutic Approaches

Several approaches aimed at blocking the neurotoxic activity of Aβ are presently pursued:

- Inhibition of amyloid production by inhibiting the enzymes cleaving βAPP (beta and gamma secretases) and thus producing Aβ. Secretase inhibition is not the focus of this review, since this has been the subject of several recent articles [32, 33].
- Immunizing against AD is a simple idea based on the immunizing principle, as is done for polio for example. Elan Pharmaceuticals [34] has recently used a transgenic mouse model of AD in which they injected a vaccine composed of β-amyloid and an immune system activating agent. The immunized mice showed virtually no plaques. Although many questions remain to be answered [35, 36], Elan has entered into the clinic to validate this approach (their vaccine "beta-block" has successfully passed a phase I study).
- Inhibition of amyloid polymerization. This approach will be more extensively developed in the following section.

B. Aβ Aggregation Inhibition

Given that increased Aβ generation, its aggregation into plaques, and the resulting neurotoxicity may lead to AD, many drug discovery approaches have targeted a slowing and/or block of that phenomenon, e.g. the aggregation process leading to the formation of the well known "amyloid plaques", also called neuritic plaques. This type of plaque can be visualized using staining dyes such as Congo Red or Thioflavin S, because of the fibrillized nature of Aβ (unlike diffuse plaques containing amyloid having a beta-pleated sheet conformation but not assembled into fibrils and invisible when using those dyes).

Aβ aggregation depends on various parameters such as pH, concentration and the incubation period in aqueous medium [21]. Some "biological chaperones" like membrane phospholipid metabolites [37], gangliosides [38], glycosaminoglycans [39, 40] for example can act in vivo as catalysts for this polymerization process. It has recently been shown that "chaperone mimetics" could inhibit the self association of Aβ. For example Neurochem Inc, a Canadian pharmaceutical company, has developed small sulfated glycosaminoglycan (GAGs) mimetics inhibiting the interaction of Aβ and naturally-occurring GAGs and displaying interesting anti-fibrillogenic properties. One of their lead compound, Alzhemed™, will enter clinical trials for Alzheimer's in partnership with H. Lundbeck A/S, a Danish pharmaceutical company. Another lead compound, Fibrillex™, designed for the treatment of systemic amyloidosis, was granted orphan drug status by the FDA in the US last year [41]. The chemical structure of these molecules has unfortunately not yet been disclosed.

C. The Aβ Polymerization Process

The polymerization process of Aβ is an event proposed to be involved in the AD disease process [42]. The hypothesis for all the "βapp-tists" working on amyloid aggregation is that *the neurotoxicity induced by this peptide is related to its aggregation state which is itself related to the conformation adopted by Aβ*. Indeed, it has been shown that the aggregation, as well as the resulting toxicity of amyloid, is directly related to the peptide's capacity to adopt a beta-sheet conformation [43]. Soluble Aβ is toxic in vitro

only when the peptide undergoes an "aging process" during which its conformation changes from an alpha helix / random coil to a beta-sheet [44]. It has been reported that this phenomenon can be catalyzed in vivo by proteins (e.g. proteoglycanes, metals, ApoE4 etc...) also localized in the senile plaque. The peptide polymerizes leading to formation of fibrils during a complex process called fibrillogenesis.

The elucidation of the fibrillogenesis process is crucial for defining appropriate drug discovery approaches dedicated to inhibit the neurotoxic effects of amyloid induced by its polymerization, catalyzed or not. Indeed, the real pathogenic entity has not yet been identified, and it is probable that some precursor species rather than the fibrils themselves may be the real neurotoxic entities! With the contribution of new technologies like Atomic Force Microscopy (AFM) new intermediates like the Aβ1-40 / Aβ1-42 protofibrils have been identified in the in vitro fibril formation process [45]. AFM is particularly useful in probing the early events in the amyloid polymerization process characterized by a slow nucleation, followed by rapid growth [46]. The group of P. Lansbury at Harvard Medical School has described Aβ aggregation as a four stages process [46]:

1. Protofibril initiation that may require about 20 Aβ molecules.

2. Protofibril elongation involving coalescence of smaller protofibrils. This step seems to be reversible, but the disassembly may occur differently than protofibril growth.

3. Protofibril to fibril transition, involving protofibril association. This step is the first that could be detected by turbidity and dye-binding assays (biophysical methods used for the search of amyloid aggregation inhibitors).

4. Fibril elongation process.

The Aβ aggregation process may even involve further stages. Indeed, Lansbury's group, using AFM, recently discovered some new ring and sphere-like structures in the α-synuclein aggregation process [47]. The protein α-synuclein is the fibrous portion of the Lewy bodies, intraneuronal cytoplasmic inclusions found in the *substancia nigra* brain region of Parkinson's patients. As some similarity can be drawn between synuclein aggregation in Parkinson's disease and amyloid aggregation in AD, it may be speculated that some very early intermediates could also be formed in the Aβ polymerization process. In fact, formation of such Aβ ring species has been evoked very recently [48], but unfortunately no published data are presently available.

It becomes obvious that if fibrils are considered to be important players regarding the toxicity induced by amyloid, inhibition of their formation should then address all potential intermediates that also may be toxic. This is a difficult task to perform, as the measurement of amyloid formation is complex, existing methods having complementary strengths and weaknesses. The common methods used in drug discovery programs related to the identification of amyloid aggregation inhibitors are: electron microscopy, turbidity, sedimentation, birefringent Congo red binding, Thioflavin-T induced fluorescence, Light Scattering, Circular Dichroism (CD), NMR and AFM [46].

The current Medicinal Chemistry approaches that target amyloid polymerization are mainly addressing the fibril formation. It is important to remember that what is linked to AD pathogenesis is the whole amyloid fibrillogenesis process and not the fibril itself [42, 49]. Drug discovery efforts should be dedicated to the design of amyloid aggregation inhibitors able to inhibit the formation of all potential intermediates as well. It then becomes clear that the α-helix / random coil to β-sheet conformational transition may constitute the target of choice. Efficacious amyloid aggregation inhibitors should be considered as "alpha helix/random coil conformational stabilizators ".

The design of amyloid aggregation inhibitors needs to consider amyloid polymerization as a protein folding problem. Most of the aggregation inhibitors reported today (see below and Fig (1) for peptide and Figs (2, 3) for non peptide inhibitors) have been discovered using a combination of biophysical methods (Electron microscopy, light scattering, CD, Fluorescence, recently AFM...) which enables the identification of how and when the molecule affects the polymerization process. This is very important for establishing a meaningful structure . activity relationship (SAR). Indeed, in order to construct a consistent SAR related to one scaffold, the molecules bearing that scaffold have to display the same binding mode to amyloid, for example. Molecules bearing a defined scaffold but interacting with different species of the fibrillogenesis pathway (i.e. with a different molecular environment) might be difficult to optimize. For example, using fluorescence assays (like the thioflavine-T assay interacting only at the stage of fibril formation) coupled to CD and better AFM (allowing detection of the molecular interaction with early formed species like the protofibril) should be of great importance to help identifying the site of action of the molecules tested.

III. AMYLOID AGGREGATION INHIBITORS IN THE DRUG DISCOVERY PROCESS

The search for potent drug like amyloid aggregation inhibitors is not very different from any other search for CNS drug candidates in terms of lead finding and optimization.

A. Lead Finding Process

The first line of in vitro assays (fluorescence type assays, microscopy) are dedicated to the identification of molecules interfering with the fibrillogenesis process (via different mechanisms that may involve the inhibition of the conformational change of Aβ). This type of assay sometimes display an important variability in the results obtained, due to the well known "batch to batch variability" of amyloid even if obtained from the same supplier [50]. It appears that reproducibility is the most important hurdle when setting-up such models. Some of these assays have been modified in order to allow high throughput screening [51].

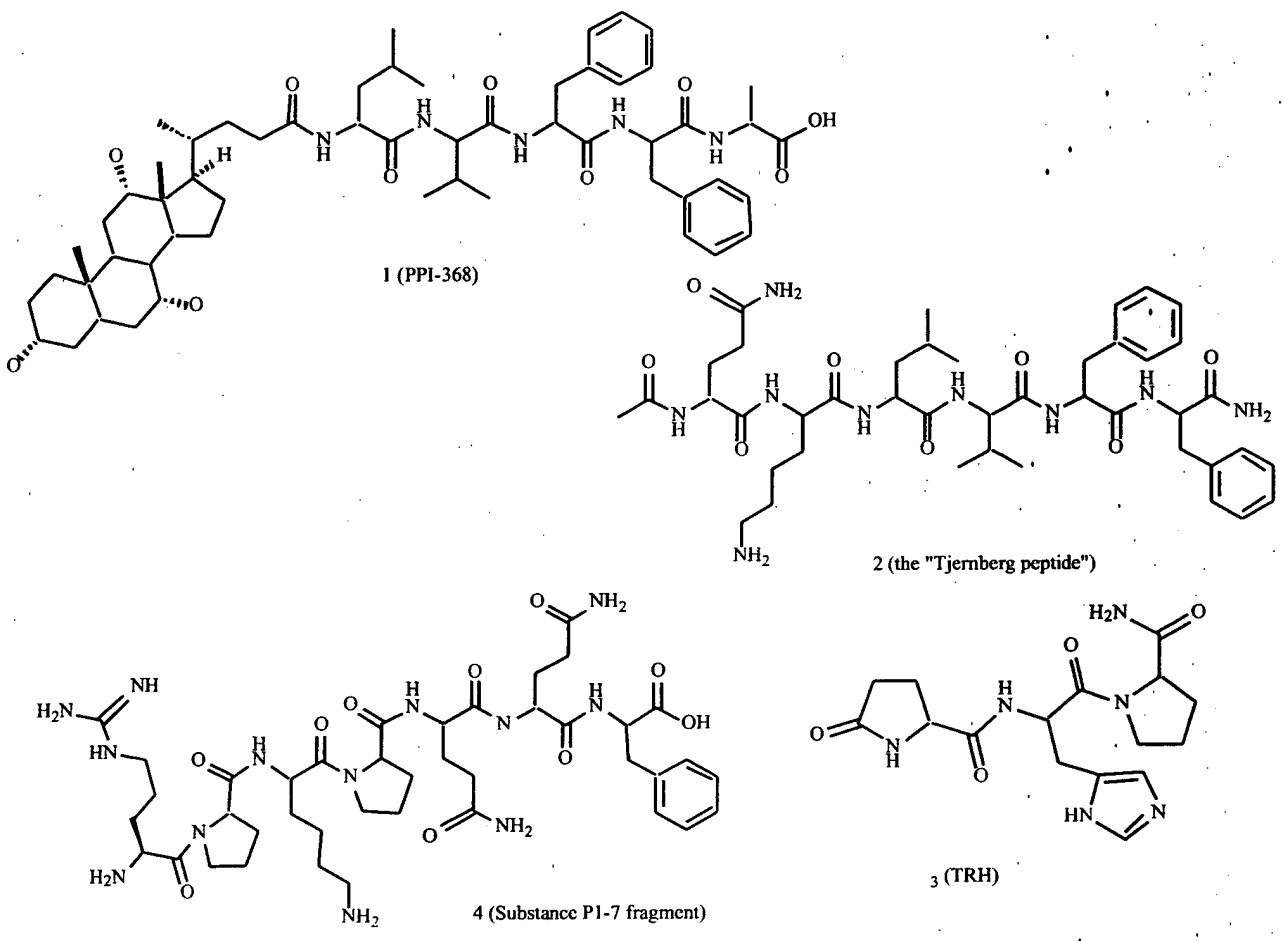


Fig. (1). Peptidic amyloid aggregation inhibitors.

The second line of in vitro assays should explore neuroprotective activity in models where amyloid is the neurotoxic agent. One of the most popular assays used in this respect is the "MTT reduction assay" [52]. Many other in vitro models are available for this purpose [52]. Once a sufficient correlation between inhibition of the fibrillogenesis process and neuroprotection has been achieved, one should directly move to lead optimization.

B. Lead Optimization Process

Lead optimization includes a certain number of well known criteria that the lead compound has to respect such as:

- A drug like scaffold (possibility of chemical modulation, synthetic accessibility,....)
- Oral activity, no toxic alert and satisfactory pharmacokinetic properties
- Efficient blood brain barrier (BBB) penetration. Two main parameters have to be considered when evaluating the transport through the BBB: the number of hydrogen bonds the molecule forms and its molecular weight [53, 54]. Integration of rules such as the "Lipinski's rules" [55], specific chemical modifications (blockade of H bond-forming functional groups, amino and/or carboxyl function directed PEGylation, see [53]] help overcome this problem. The BBB penetration will generally be evaluated in vivo via microdialysis experiments.
- In vivo efficacy and potency

To date, literature is scarce with regard to preclinical as well as clinical data related to amyloid aggregation inhibitors. Parameters related to appropriate pharmacokinetic and toxicity properties could be addressed using approaches routinely employed for the evaluation of CNS related drug candidates. In contrast, a major obstacle will relate to characterizing appropriate pharmacodynamic properties of an amyloid aggregation inhibitor before it enters into clinical testing. Efficacy should optimally be evaluated using relevant in vivo animal models reproducing the hallmarks of AD. Unfortunately, no such model exists presently. Nevertheless, "incomplete" models can be divided into three general classes [56] which may support the therapeutic utility of those molecules:

- Classical cognitive models involving "normal" aged animals (mice, rat, monkeys, dogs) in traditional experimental paradigms of learning and memory, having the advantage of being physiologically integrated approaches of normal aging, and the disadvantage of not specifically reflecting the pathology of AD.

- Neurodegeneration models especially the β-amyloid toxicity models using icv injection of "aged" Aβ into rats [57-59] thereby altering cognitive performance in several models like the water maze task, or the social recognition test. However such murine models are subject to controversy, since amyloid injections not always induce an Alzheimer like pathology [60]. A more promising model appears to be the aged monkey model (Rhesus monkey) in which icv injections of fibrillar Aβ [61] induce neuronal loss, tau hyperphosphorylation (another hallmark of AD, [62]] as well as microglial proliferation.

- Transgenic models involving generally gene overexpression in mice (mutated human APP) or Knockout-mice (APP null mutation) [63, 64].

Animal models presenting both the histopathological features and memory deficits characterizing AD unfortunately do not exist. Moreover, those proposed presently remain excessively costly and thereby difficult to use for a routine purpose. Only selected lead candidates may be tested in advanced models like the transgenic models displaying cognitive deficits. Indeed, amyloid aggregation inhibitors, beside showing a decrease in plaque formation, should display cognitive enhancement in vivo, in order to substantiate the investment into clinical trials. Clinicians are not mainly concerned with the mechanism of action of a drug, but more whether it holds a promise to reduce or abolish the progression of AD. Clearly, treatment can be broadly divided into two categories: agents improving symptoms especially cognitive function and those that decrease the progression of the disease. Inhibition of amyloid aggregation is obviously expected to be part of the second category of therapeutic agents, but this may be difficult and costly to demonstrate in clinical trials. However a correlation between the decrease in amyloid plaque formation and an amelioration in cognitive performance (the only non-invasive "measurable" criterion in man today) should dramatically boost the clinical development of such agent.

C. Amyloid Aggregation Inhibitors: Where are We Today?

Molecules inhibiting the polymerization process of Aβ have been discovered via two main strategies: random screening, or based on amyloid short peptide sequences inhibiting amyloid aggregation.

1. Peptidic Amyloid Aggregation Inhibitors, Fig (1).

It has been reported that the ability of Aβ to successively assume a β-pleated sheet conformation and to form fibrils is dependent on the hydrophobic sequence KLVFF (Aβ16-Aβ20) [65]. This observation leads to peptidic amyloid aggregation inhibitors like:

- peptides having the LVFF sequence coupled to a steroid moiety at its N-terminus e.g. **PPI-368, 1** [66]. This compound is apparently able to block the formation of all neurotoxic species of Aβ oligomers. The steroid nucleus has no effect per se. It seems that PPI-368 binds directly to amyloid monomers or soluble oligomers.

- pentapeptides bearing the complete KLVFF sequence (like **AcKLVFFNH$_2$, 2**) which are able to inhibit the assembly of full length Aβ into fibrils [67]. Using combinatorial pentapeptide libraries, Tjernberg et al found that peptides composed of D-amino acids also selectively inhibit amyloid fibril formation [68, 69]. Peptides bearing the **lflrr** or **yflrr** sequence (D-amino-acids) completely blocked the fibril formation. Unfortunately, the literature appears devoid of data regarding the testing of such peptides using in vitro/vivo amyloid induced neurotoxicity assays. Molecular modeling studies (docking between KLVFF and Aβ13-23) indicate an anti-parallel arrangement in which residues Lys and Leu in KLVFF interact with Phe20 in the homologous sequence; the D-amino-acid peptides forming a parallel β-sheet [69]. The same group also recently published an interesting molecular model of amyloid fibril formation [70] underlying an anti-parallel β-sheet conformation adopted by the Aβ14-23 oligomers, allowing favorable hydrophobic interactions as well as stabilization via salt bridges between all charged residues. Their last publication, together with Hoffmann-La Roche [71] describes non peptidic amyloid aggregation enhancers as well as inhibitors based on a **pyridone scaffold 5, 6**, Fig 2. These molecules were identified by random high throughput screening. Another patent from the same group [72] claimed molecules bearing such pyridone scaffold as potent inhibitors of amyloid production.

- Longer peptides still bearing a "KLVFF sequence" (recognition element) combined to an oligolysine disrupting element, e.g. **QKLVFFAEDVGG*a*KKK KKK** (*a* for "aminocaproate" linker, 73). This molecule has interesting characteristics. It causes subtle changes in the aggregation kinetics, the fibril morphology and inhibits the in vitro neurotoxicity of Aβ1-39 in the MTT reduction test [73]. This neuroprotective effect did apparently not require measurable conformational changes, nor the prevention of fibril formation. Shorter compounds like **KLVFF-KKKKKK** were also neuroprotective (without aggregation prevention) indicating the importance of this pentapeptide motif which should be a very good starting point for the design of non-peptidic inhibitors of amyloid toxicity [74].

- Peptides bearing an LPFFD sequence, according to experimental data showing that Proline replacement in any residue in an amyloidogenic sequence like LVFFAED results in a loss of fibril formation [75].

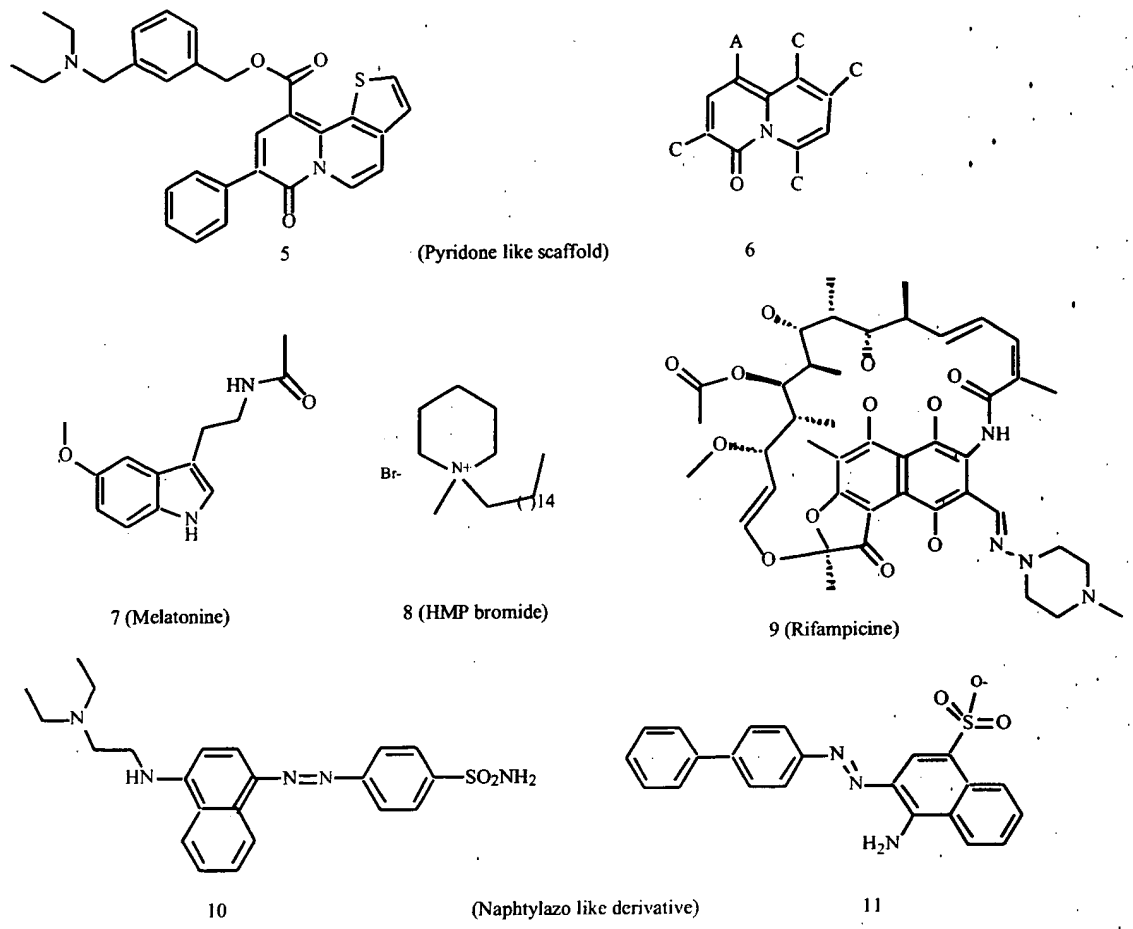


Fig. (2). Non peptide Amyloid aggregation inhibitors.

Peptidyl-proline bonds being incapable of forming standard extended chain conformations. Soto's group reported the synthesis of peptides containing the "masked" KLVFF sequence, for example **LPFFD** (Iab5) and **RDLPFFPYPID** (Iab11) which inhibit amyloid fibrillogenesis [76]. Moreover these compounds dissolved pre-formed Aβ fibrils. The pentapeptide Iab5 has been reported [77] to display neuroprotective activity in vitro (human neuroblastoma cells) as well as in vivo (icv injection of LPFFD in Aβ1-42 treated rats showed smaller amyloid deposits near the injection site). These peptides are claimed to represent novel therapeutic approaches for AD and prion disease [78]. Unfortunately, it is well known that problems like rapid metabolism, poor BBB penetration hamper the use of peptide derivatives as drugs. Soto's group tried to improve those parameters by coupling polyamine chains like putrescine to the active all d-enantiomer of Iab11. The results obtained involved a 5 to 7 fold increase of BBB penetration and complete protection from degradation by rat plasma protease which appears encouraging [79].

We performed a comparative in vitro study of three representatives of the above mentioned peptides [80]. The study was designed to evaluate the anti-aggregation and neuroprotective properties of: KLVFF ("Tjernberg peptide"), GQKLVFFAEDVGGaKKKKKK ("Ghanta peptide") and RDLPFFPVPID ("Soto peptide"). $A\beta_{1-40}$ aggregation was assessed by thioflavin-T fluorescence spectroscopy, circular dichroism as well as a light scattering assay. $A\beta_{1-40}$ toxicity in PC12 cells was evaluated using the MTT reduction assay. KLVFF was identified as the most potent antiaggregatory peptide : ThT fluorescence was inhibited by 50%(IC50 7 μM) and β-sheet content was 20% (43% for Aβ alone). PC12 cells were completely protected against Aβ(100 nM) toxicity at 1:1 and 1:0.5 Aβ:KLVFF molar ratios. The "Ghanta peptide" and the "Soto peptide" were less effective (10-25%) at reducing β-sheet content and ThT fluorescence (IC50 values of 90 and 75 μM, respectively). The "Ghanta peptide" protected PC12 cells at 1:0.5 and 1:0.25 molar ratios. Our results demonstrate that KLVFF displays the most potent anti-aggregation - neuroprotective effects among the three reference peptides tested.

A. Thyrotrophin releasing hormone (TRH) and Substance P

TRH Mimetics as Aβ Aggregation Inhibitors?

Scientists from Eli Lilly recently reported that the tripeptide TRH, **pGluHisPro-NH$_2$**, **3**, was able to inhibit

the neurotoxic effect of Aβ1-40 in the MTT reduction assay [81]. Unfortunately no data regarding a potential inhibition of the fibrillogenesis process were reported. TRH peptidomimetics as potential inhibitors of amyloid aggregation would represent an exciting new area of drug design, knowing that such compounds have previously been reported to enhance cognitive function [82-84].

Substance P Mimetics as Aβ Aggregation Inhibitors?.

The substance P peptide (SP: ArgProLysProGlnGlnPhe PheGlyLeuMet-NH_2), is a well known pro-inflammatory peptide acting via the NK1, NK2 and NK3 receptors [85]. Neurokinin antagonists, are presently developed in several therapeutic indications like asthma, pain, migraine, emesis and depression. It has been reported that the in vitro [86] as well as the in vivo [87] neurotoxicity induced by Aβ can be antagonized by SP related peptides. Very interestingly, these effects can be mimicked by the N-terminal fragment of SP e.g. SP1-7: ArgProLysProGlnGlnPhe, **4**, which does not bind to the neurokinin receptors, and is consequently not prone to induce inflammation [88]. It may be possible that SP directly interacts with the polymerization process of Aβ. Unfortunately, some studies did not succeed in reproducing this observation [15, 89]. Nevertheless, another recent patent [90] claimed the use of tachykinin agonists as effective inhibitors of amyloid induced neurotoxicity. Moreover, a promnestic effect of SP has been reported and is claimed to be mediated via it's N-terminal part [91]. It therefore appears that non peptidic SP-N-terminal fragment mimetics could constitute an original and viable drug discovery approach related to amyloid aggregation inibition coupled to cognition enhancement activity.

2. Non Peptidic Amyloid Aggregation Inhibitors, Figs (2) & (3).

- **Melatonin, 7**, Fig (2), beside its well known anti-oxidant / free radical scavenger properties [92] has also been reported to inhibit Aβ1-40 and Aβ1-42 fibril formation [93].

- **Hexadecyl-N-methylpiperidinium (HMP) bromide, 8**, Fig (2), has been reported to inhibit the in vitro Aβ1-40 fibril formation [94]. This molecule has been identified via screening.

- **Rifampicin, 9**, Fig (2), the well known antibiotic inhibits the aggregation as well as the in vitro neurotoxicity (PC12 cells) induced by Aβ1-40, apparently via a radical scavenging mechanism of action [95].

- **Naphtylazo derivatives, 10**, Fig (2) of the dye Congo Red, which inhibits amyloid fibril formation, have been reported by Warner Lambert [96]. A molecular modeling model for structure-dependent binding of Congo Red to amyloid fibrils was published some years ago [97]. Scientists from Eli Lilly also worked on an azo-dye scaffold [98] represented by compound **11**, Fig (2) showing a correlation between the inhibition of Aβ1-40 neurotoxicity and fibril growth. Such molecules could also be used as a tool to measure amyloid deposits in vivo. Indeed, naphtylazo derivatives complexed to technetium(V) have been proposed as potential reagent(s) for SPECT (Single Photon Computed Tomography) imaging of AD brains [99].

- Some other agents have also been suggested as potential therapeutic inhibitors of amyloid aggregation e.g. **anthracyclone derivatives 12**, Fig (3) synthesized by Pharmacia & Upjohn [100]. One representative molecule of that family **IDX** (4'-Deoxy-4'-iododoxorubicin, **13**, Fig (3) inhibits seed triggered amyloid 1-40 aggregation into fibrils. Activity can be correlated with the compound's lipophilicity [101]. It is active in experimental models of prion amyloidosis. A molecular modeling study reporting the docked structure of IDX with a model of amyloid fibrils has been recently published [102].

- A series of **benzofuran derivatives** (e.g. **SKF-74652, 14**, Fig (3)) have been claimed by SmithKline Beecham to inhibit the fibrillogenesis of Aβ1-40 as well as to inhibit MTT reduction induced by amyloid fibrils [103]. This scaffold, also patented by Lilly [104] seems to be one of the first really drug like scaffold in terms of lead optimization.

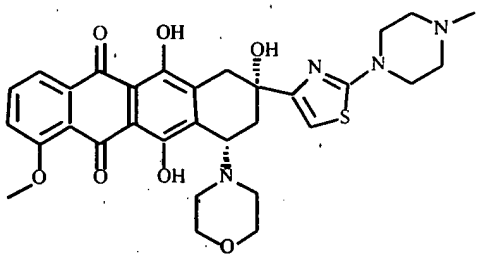
- Gerolymatos has claimed the use of **phanquinone, 15**, Fig (3), as an inhibitor of metal (Zn^{++} and Cu^{++}) induced amyloid aggregation [105].

- Beta-sheet nucleating peptidomimetics bearing a **diaryl heterocyclic, 16**, Fig (3), scaffold have been claimed as amyloid aggregation inhibitors by Kelly [106]. Unfortunately no suitable biological data were given. Other heterocyclic molecules such as the **9-acridone derivatives, 17**, Fig (3), seem also to inhibit the amyloid polymerization process [107].

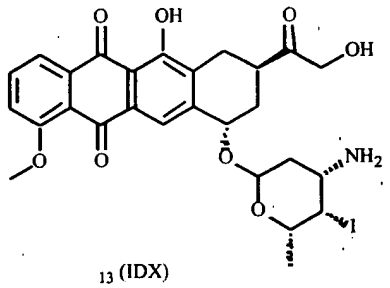
- Centaur Pharmaceuticals has recently described some interesting **furansulfonic acid derivatives, 18**, Fig. (3), able to inhibit the formation of Aβ(1-40) beta-pleated sheets and to protect against neuronal cell loss induced by an Aβ(25-35) fragment, as well as to inhibit the production of pro-inflammatory cytokines (e.g. IL-1β, TNFα) induced by amyloid [108]. Additionally, some derivatives have been found to reduce the in vivo locomotor impairment caused by Aβ(25-35), as well as to reduce the cognitive deficits that develop in certain strains of autoimmune mice [108]. This class of molecules belongs to the few which have been reported to display in vivo activity in cognition related models!

3. Are There Any "Drug Like" Amyloid Aggregation Inhibitors Available Today?

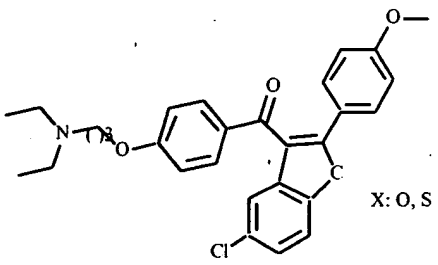
Non peptidic amyloid aggregation inhibitors are generally flat molecules bearing a lipophilic bi- or tri-(hetero)cyclic scaffold (2-4 phenyl rings) and having a basic nitrogen on one of these rings. Such conjugated poly-aromatic derivatives may not display an ideal drug like



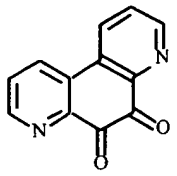
12 (anthracyclone derivative)



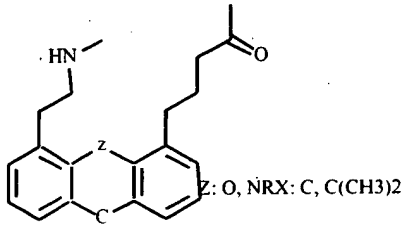
13 (IDX)



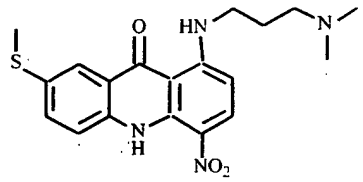
14 (benzofurane derivative)



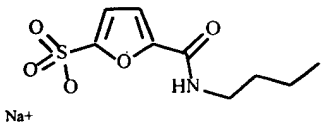
15 (phanquinone)



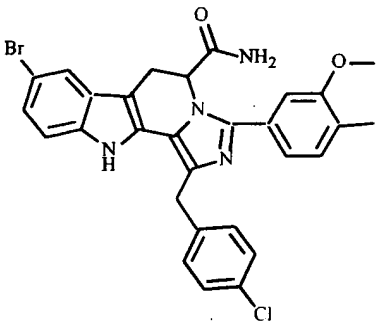
16 (diaryl heterocyclic derivative)



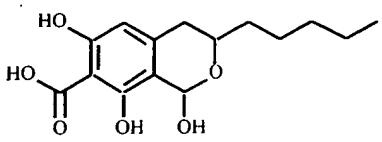
17 (9-acridone derivative)



18 (furansulfonic acid derivative)



19 (imidazopyridoindole derivative)



20 (isochroman derivative)

Fig. (3). Non peptide Amyloid aggregation inhibitors (contd.).

profile. One exception is the "atypical Centaur molecule", a sulfonic acid furan derivative, which may bind to amyloid in a completely different manner. An ideal drug has to be orally active, readily absorbed from the gut, and has to reach its target in this case the brain by readily crossing the BBB. The molecule should then be metabolized in the liver before being excreted. Although many tools have been developed in order to address these problems (see ref. [109] for an update), very few data addressing solubility, lipophilicity, molecular weight and other properties influencing an amyloid aggregation inhibitor's pharmacokinetic and toxicological profile, have yet been published. Ignoring these "drug development issues" at the beginning of the lead finding stage will obviously increase the time frame of the whole drug discovery process. This may explain why no real drug like amyloid aggregation inhibitors have been published so far.

IV. CONCLUSION

The way to make the dream of Aβ aggregation inhibitors for treating AD become reality is still long and hard. First of all, Aβ is necessary but certainly not sufficient for producing

the features of the pathology of AD. The greatest risk factor for the development of AD still remains age! Amyloid might even be of benefit, restoring some degree of cognition! [110]. Moreover, amyloid aggregation can be studied in vitro using methodologies becoming more and more sophisticated, but the relevance for the in vivo condition still remains obscure. The same limitation relates to the in vitro neurotoxic assays used to predict a neuroprotective effect of amyloid aggregation inhibitors. The recent discovery by Benveniste et al [111] that magnetic resonance microscopy constitutes a methodology of choice for the detection of neuritic plaques in vitro and potentially in vivo, opens an exciting avenue for evaluating the in vivo activity of amyloid aggregation inhibitors in transgenic mice. This may supply us with a more realistic assessment of the *in vivo* potential of such a strategy and its potential for extension to other "conformational diseases" [112] where abnormal protein folding and then aggregation may play a fundamental pathogenic role.

The clinical trials methodology necessary to demonstrate a neuroprotective/symptomatic (cognitive) activity of amyloid aggregation inhibitors will have to be innovative, since no clinical experience with this approach is available at present. Monitoring of CSF markers like amyloid or tau proteins will be hampered by the difficulty of enrolling patients willing to undergo lumbar puncture. Neuroimaging measures of brain atrophy may provide an alternative to this invasive methodology [113]. Moreover, pharmacogenomics will enable the selection of a more targeted population, thus allowing the development of a "custom tailored drug". This concept has already been applied to some AD drugs like Tacrine (acetylcholine esterase inhibitor) that appears more efficacious in patients having the ApoE E2 and ApoE E3 allele than in those carrying the ApoE E4 [114]. Although this assumption has been challenged very recently by Pfizer [115]. On the other hand, Sanofi's S12024 (whose mechanisms of action target the noradrenergic / vasopressinergic systems and not the cholinergic system) appears to induce a superior activity in patients having the ApoE E4 genotype [114].

Several problems need to be solved before the therapeutic value and utility of amyloid aggregation inhibitors can be established. However, the amyloid beta peptide remains a rational and viable therapeutic target [116], that holds the potential of being the first real efficacious treatment for Alzheimer's disease.

ADDENDUM

During the publication process of this article, other amyloid aggregation inhibitors have been reported in the literature. The Torrey Pines Institute described a mixture-based synthetic combinatorial library composed of 23375 imidazopyridoindole derivatives [117]. Compound **19**, Fig (3), the most potent amyloid (Aβ25-35) aggregation inhibitor (IC_{50}: 42+/-7 μM) showed neuroprotective activity against Aβ1-42 induced toxicity in PC12 cells. Interestingly, **19** inhibited the random coil to β-sheet transition and self aggregation of the amyloid peptide. Among the pharmaceutical companies involved in this type of research, Pfizer reported the isolation of an isochroman compound, **20**, Fig (3), produced by fermentation of a fungus, *Penicillium simplicissimum*, which has been found to inhibit Aβ1-40 protein aggregation [118].

The quest for the ideal drug-like amyloid aggregation inhibitor remains a difficult task, since the processes by which Aβ aggregates are complex and still poorly understood. Amyloid nucleation, fibril formation, growth of non fibrillar material may all be implicated in the aggregation process, with each amyloid oligomeric and/or polymeric forms potentially inducing different neurotoxic processes. As has been reported recently, Aβ oligomers (dimers) may constitute the real toxic entities killing the cell via "channel-mediated" toxicity [119, 120]. Most importantly, a "proof of concept" study using genetically-engineered mice overproducing amyloid will help to define the best strategy as well as the curative potential of this approach. Such a study has recently been published for the first time by the Mayo Clinic [121], using the Tg2576 animal model [122]. The therapeutic approach tested, however, was not related to amyloid aggregation but to inhibition of extracellular Aβ accumulation using wortmanin (a phosphatidyl-inositol kinase inhibitor) to decrease plaque formation. Unfortunately a correlation between plaque formation decrease and cognitive effects was not reported. Nevertheless, the results with the first non-toxic, drug-like Aβ aggregation inhibitor in such a model are eagerly awaited.

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LIST OF ABBREVIATIONS

AD	=	Alzheimer's Disease
NSAIDs	=	Non Steroidal Anti Inflammatory Drugs
Aβ	=	Amyloid peptide
APP	=	Amyloid Precursor Protein
PS	=	Presenilin
GAGs	=	Glycoaminoglycans
AFM	=	Atomic Force Microscopy
CD	=	Circular Dichroism
SAR	=	Structure Activity Relationship
MTT	=	(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
BBB	=	Blood Brain Barrier

CSF = Cerebro Spinal Fluid

TRH = Thyrotrophin Releasing Hormone

SPECT = Single Photon Computer Tomography

PS = Presenilin

ApoE = Apolipoprotein E

V. REFERENCES

[1] Hebert LE.; Scherr PA.; Becket LA.; et al. *JAMA* **1995**, *273*, 1354.

[2] Evans DA.; Funkenstein HH.; Albert MS. *JAMA* **1989**, *262*, 2551.

[3] Mayeux R.; Sano M. *New England J. Medicine* **1999**, *341*, 1670.

[4] Francis PT.; Palmer AM.; Snape M.; Wilcok. *J. Neurol. Neurosurg. Psychiatry*. **1999**, *66*, 137.

[5] Emilien G.; Beyreuther K.; Masters CL.; Maloteaux JM. *Arch. Neurol.* **2000**, *57*, 454.

[6] Jenkinson ML.; Bliss MR.; Brain AT.; Scott DL. *Br. J. Rheumatol.* **1989**, *28*, 86.

[7] McGeer PL.; McGeer EG. *Dementia* **1995**, *9*, 111.

[8] Oken BS.; Storzbach DM.; Kaye JA. *Archives of Neurology* **1998**, *55*, 1409.

[9] Targum SD.; Wieland S.; Glasky MS.; Glasky AJ. *Eur. Neuropsychopharmacol.* **1999**, *9*, Suppl. 5, S320.

[10] Selkoe DJ. *J. Biol. Chem.* **1996**, *271*, 18295.

[11] Haass C.; Hung AY.; Schlossmacher MG.; Teplow DB.; Selkoe DJ. *Nature* **1992**, *359*, 322.

[12] Whitson JS.; Selkoe DJ.; Cotman CW. *Science* **1989**, *243*, 1488.

[13] Kowall NW.; McKee AC.; Yankner BA.; Beal MF. *Neurobiol. Aging* **1992**, *13*, 537.

[14] Emre M.; Geula C.; Ransil BJ.; Mesulam MM. *Neurobiol. Aging* **1992**, *13*, 553.

[15] Rush DK.; Aschimies S.; Merriman MC. *Neurobiol. Aging* **1992**, *13*, 591.

[16] Busciglio J.; Lorenzo A.; Yankner BA. *Neurobiol. Aging* **1992**, *13*, 609.

[17] Pike CJ.; Burdick D.; Walencewicz AJ.; Glabe CG.; Cotman CW. *J. Neurosci.* **1993**, *13*, 1676.

[18] Lorenzo A.; Yankner BA. *Proc. Natl. Acad. USA* **1994**, *91*, 12243.

[19] Yatin SM.; Varadarajan S.; Link CD.; Butterfield DA. *Neurobiol. Aging* **1999**, *20*, 325.

[20] Mattson MP.; Barger SW.; Cheng B.; Liederburg I.; Smith-Swintotosky W.; Rydel RE. *TINS* **1993**, *16*, 409.

[21] McKeon-O Malley C.; Saunders AJ.; Bush AI.; Tanzi RE. *Emerging Therapeutic Targets* **1998**, *2*, 157.

[22] Mantyh PW.; Ghilardi JR.; Rogers S.; DeMaster E.; Allen CJ.; Stimson ER.; Maggio JE. *J. Neurochem.* **1993**, *61*, 1171.

[23] Allsop D.; Williams CH. *Biochem. Soc. Trans.* **1994**, *22*, 171.

[24] Nitsch RM.; Growdon JH. *Biochem. Pharmacol.* **1994**, *47*, 1275.

[25] Steiner H.; Capell A.; Haass C. *Biochem. Soc. Trans.* **1999**, *27*, 234.

[26] Kimberly WT.; Xia W.; Rahmati T.; Wolfe MS.; Selkoe DJ. *J. Biol. Chem.* **2000**, *275*, 3173.

[27] De Stooper B. *Nature* **2000**, *405*, 627.

[28] Selkoe DJ.; Wolfe MS. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5690.

[29] Vassar R.; Bennett BD.; Babu-Khan S.; Kahn S.; Mendiaz EA.; Denis P.; Teplow DB.; Ross S.; Amarante P, Loedloff R.; Luo Y.; Fisher S.; Fuller J.; Edenson S.; Lile J.; Jarosinski MA.; Biere AL.; Curran E.; Burgess T.; Louis JC.; Collins F.; Treanor J.; Rogers G.; Citron M. *Science* **1999**, *286*, 735.

[30] Sinha S.; Anderson JP.; Barbour R.;Basi GS.; Caccavello R.; Davis D.; Doan M.; Dovey HF.; Frigon N.; Hong J.; Jacobson-Croak K.; Jewett N.; Keim P.; Knops J.; Liederburg I.; Power M.; Tan H.; Tatsuno G.; Tung J.; Schenk D.; Seubert P.; Suomensaari SM.; Wang S.; Walker D.; Zhao J.; McConlogue L.; WargheseJ. *Nature* **1999**, *402*, 537.

[31] Rosenberg RN. *Neurology* **2000**, *54*, 2045.

[32] Augelli-Szafran CE.; Walker LC.; LeVine III H. *Annual Rep. Med. Chem.* **1999**, 21.

[33] Moore CL.; Wolfe MS. *Exp. Opin. Ther. Patents* **1999**, *9*, 135.

[34] Schenk D.; Barbour R.; Dunn W.; Gordon G.; Grajeda H.; Guido T.; Hu K.; Huang J.; Johnson-Wood K.; Khan K.; Kholodenko D.; Lee M.; Liao Z.; Liederburg I.; Mutter L.; Soriano F.; Shopp G.; Vasquez N.; Vandevert C.; Walker S.; Wogulis M.; Yednock T.; Games D.; Seubert P. *Nature* **1999**, *400*, 173.

[35] Blass JP. *The New Engl. J. Medicine* **1999**, *341*, 1694.

[36] Duff K. *TINS* **1999**, *22*, 485.

[37] Klunk WE.; Xu CJ.; McClure RJ.; Panchalingam K.; Stanley JA.; Pettegrew. *J. Neurochem.* **1997**, *69*, 266.

[38] Choo-Smith LP.; Garzon-Rodriguez W.; Glabel CG.; Surewicz WK. *J. Biol. Chem.* **1997**, *272*, 22987.

[39] Brunden KR.; Richter-Cook NJ.; Chaturvedi N.; Frederickson RCA. *J. Neurochem.* **1993**, *61*, 2147.

[40] McLaurin J.; Franklin T.; Zhang X.; Deng J.; Fraser PE. *Eur J. Biochem.* **1999**, *266*, 1101.

[41] *SCRIP* **2000**, *2561*, 14.

[42] Selkoe DJ. *Science* **1997**, *275*, 630.

[43] Kirschner DA.; Abraham C.; Selkoe DJ. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 503.

[44] Simmons LK.; May PC.; Tomaselli KJ.; Rydel RE.; Fuson KS.; Brigham EF.; Wright S.; Liedeburg I.; Becker GW.; Brems DN.; Li WY. *Molecular Pharmacol.* **1994**, *45*, 373.

[45] Harper DH.; Wong SS.; Lieber CM.; Lansbury Jr PT. *Biochemistry* **1999**, *38*, 8972.

[46] Lansbury PT.; Harper JD. *Annu. Rev. Biochem.* **1997**, *66*, 385.

[47] Conway KA.; Lee SJ.; Rochet JC.; Ding TT.; Williamson RE.; Lansbury PT Jr. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 571.

[48] Perry G. *Idrugs* **2000**, *3(5)*, 485.

[49] Lansbury PT. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 3342.

[50] Snow AD.; Malouf AT. *Hippocampus* **1993**, 3, special issue, Eds R. Nitsch and TG Ohm, 257-268.

[51] Esler WP.; Stimson ER.; Ghilardi JR.; Felix AM.; Lu YA.; Vinters HV.; Mantyh PW.; Maggio JE. *Nature Biotech.* **1997**, *15*, 258.

[52] Iversen LL.; Mortishire-Smith RJ.; Pollack SJ.; Shearman MS. *Biochem J.* **1995**, *311*, 1.

[53] Pardridge WM. *J. Neurochem.* **1998**, *70*, 1781.

[54] Emerich DF. *Exp. Opin. Ther. Patents.* **2000**, *10*, 279.

[55] Lipinski CA. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3.

[56] Allain, H.; Bentué-Ferrer, D.; Zekri O.; Schück S.; Lebreton S.; Reyman JM. *Fundam. Clin. Pharmacol.* **1998**, *12*, 13.

[57] Nitta A.; Itoh A.; Hasegawa T.; Nabeshima T. *Neurosci. Lett.* **1994**, *170*, 63.

[58] Terranova JP.; Kan JP, Storme JJ.; Perreaut P.; Le Fur G.; Soubrie P. *Neurosci. Lett.* **1996**, *213*, 79.

[59] Nabeshima T.; Nitta A. *Tohoku J. Exp. Med.* **1994**, *174*, 241.

[60] Games D.; Khan KM.; Soriano FG.; Keim PS.; Davis DL.; Bryant K.; Liedeburg I. *Neurobiol. Aging* **1992**, *13*, 569.

[61] Geula G.; Wu CK.; Saroff D.; Lorenzo A.; Yuan M.; Yankner B. *Nature Medicine* **1998**, *4*, 827.

[62] Tolnay M.; Probst A. *Neuropathol. Appl. Neurobiol.* **1999**, *25*, 171-187.

[63] Sirinathsinghji DJS. *Biochem. Soc. Trans.* **1998**, *26*, 504.

[64] Guénette SY.; Tanzi RE. *Neurobiol. Aging* **1999**, *20*, 201.

[65] Hilbich C.; Kisters-Woike B.; Reed J.; Masters CL.; Beyreuther K. *J. Mol. Biol.* **1992**, *228*, 460.

[66] Molineaux SM.; Chin J.; Lee JJ.; Kelley M.; Kubasek W.; Wakefield J. *26th Annual Meeting Society for Neuroscience, Washington DC, USA*, 16-21 November **1996**. Poster, 651.1

[67] Tjernberg LO.; Naslund J., Lindqvist F et al. *J. Biol. Chem.* **1996**, *271*, 8545.

[68] Nordstedt C.; Torg N.; Näslund J.; Thyberg J.; Tjernberg LO.; Terenius L. *Patent application* WO 97/21728, **1997**.

[69] Tjernberg LO.; Lilliehöök.; Callaway JE.; Näslund J.; Hahne S.; Thyberg J.; Terenius L.; Nordsted C. *J. Biol. Chem.* **1997**, *272*, 12601.

[70] Tjernberg LO.; Callaway DJE.; Tjernberg A.; Hahne S.; Lilliehöök C.; Terenius L.; Thyberg J.; Nordstedt C. *J. Biol. Chem.* **1999**, *274*, 12619.

[71] Kuner P.; Bohrman B.; Tjernberg LO.; Näslund J.; Huber G.; Celenk S.; Grüninger-Leicht F.; Richards JG.; Jakob-Roetne R.; Kemp JA.; Nordstedt C. *J. Biol. Chem.* **2000**, *275*, 1673.

[72] Trottmann H.; Jakob-Roetne R.; Kolczewski S.; Norcross RD.; Woltering TJ. *Patent application* N° WO98/25930, **1998**.

[73] Ghanta J.; Shen CL.; Kiessling LL.; Murphy RM. *J. Biol. Chem.* **1996**, *271*, 29525.

[74] Pallitto MM.; Ghanta J.; Heinzelman P.; Kiessling LL.; Murphy RM. *Biochemistry* **1999**, *38*, 3570.

[75] Wood SJ.; Wetzel R.; Martin JD.; Hurle MR. *Biochemistry* **1995**, *34*, 724.

[76] Soto C..; Kindy MS.; Baumann M.; Frangione B. *Biophys. Res. Commun.* **1996**, *226*, 672.

[77] Soto C.; Sigurdsson EM.; Morelli L.; Kumar RA.; Castano EM.; Frangione B. *Nat. Med.* **1998**, *4*, 822.

[78] Soto C. *J. Mol. Med.* **1999**, *77*, 412.

[79] Poduslo JF.; Curran GL.; Kumar A.; Frangione B.; Soto C. *J. Neurol.* **1999**, *39*, 371.

[80] Lorent G.; Barthelemy X.; Houssier C.; Vandenplas C.; Segal J.; Talaga P.; Klitgaard H. *Neuroscience meeting communication*, **2000**, New Orleans, USA.

[81] Lilly & Co. *Patent application* N° WO9728184, **1997**.

[82] *Drugs Fut.* **1993**, *18*, 483.

[83] *Drugs Fut.* **1993**, *18*, 259.

[84] Moeller KD.; Rothfus SL. *Tetrahedron Lett.* **1992**, *33*, 2913.

[85] Otsuka M.; Yoshioka K. *Physiol. Rev.* **1993**, *73*, 229.

[86] Yankner BA.. Duffy LW, Kirschner DA. *Science* **1990**, *250*, 279.

[87] Kowall NW.; Beal MF.; Busciglio J.; Duffy LK.; Yankner BA. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 7247.

[88] Yankner BA. WO *patent application* N° 92/02248, **1992**.

[89] Bell C.; Davis J.; Cole GM.; Schubert D. *Biochem. Biophys. Res. Commun.* **1992**, *186*, 944.

[90] Yankner BA. US *patent application* N°5,876,948; **1999**.

[91] Huston JP.; Hasenohrl RU.; Gerhardt P.; Schwarting RKW. *Psychopharmacol.* **1993**, *112*, 147.

[92] Tan X.; Chen LD.; Poeggeler B.; Manchester LC.; Reiter RJ. *Endocr. J.* **1993**, *1*, 57.

[93] Pappolla M.; Bozner P.; Soto C et al. *J. Biol. Chem.* **1998**, *273*, 7185.

[94] Wood SJ.; Mac Kenzie L.; Maleef B.; Hurle MR.; Wetzel. *J. Biol. Chem.* **1996**, *271*, 4086.

[95] Tomijama T.; Asano S.; Suwa Y et al. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 76.

[96] Hays SJ.; LeVine H III.; Scholten JD. *US patent* **2000**, N°6,017,913.

[97] Carter DB.; Chou KC. *Neurobiol. Aging* **1998**, *19*, 37.

[98] Porter WJ.; Audia JE.; Boggs LN.; Droste JJ.; Fuson KS.; Hyslop PA. *215th American Chem. Soc. Nat. Meet. Dallas, Texas, USA*, 29 March-2 April **1998**. Lecture, MEDI 212.

[99] Zhen W.; Han H.; Anguiano M.; Lemere CA.; Gho CG.; Lansbury PT. *J. Med. Chem.* **1999**, 42, 2805.

[100] Mantegani S.; Traquandi G.; Bandiera T.; Lansen J.; Varasi M. *Patent application* N° WO9946253, **1999**.

[101] Bandiera T.; Caruso M.; Cavanus S.; Della VF.; Gerna M.; Lansen J. Anthracyclines as inhibitors of Abeta-peptide aggregation. *XVth International Symposium on Medicinal Chemistry, Edinburgh, Scotland 6-10 September* **1998**, poster P92.

[102] George AR.; Howlett DR. *Biopolymer* **1999**, *50*, 733.

[103] Howlett DR.; Perry AE.; Godfrey F.; Swatton JE.; Jennings KH. *Biochem J.* **1999**, *340*, Part 1, 283.

[104] Lunn WH.; Monn JA.; Zimmerman DM. *US patent application* N° 5,552,426; **1996**.

[105] Xilinas M.; Gerolymatos PN. *Patent application* N° WO9909981, **1999**.

[106] Kelly JW. *Patent application* N° WO9746547, **1997**.

[107] Hays SJ.; Levine H.; Scholten JD. *Patent application* N° WO9716191, **1997**.

[108] Kelleher JA.; Maples KR.; Zhang YK. *Patent application* N° WO99/09022, **1999**.

[109] Brennan MB. *C&EN* **2000**, *5*, 63.

[110] Perry G.; Nunomura A.; Raina AK.; Smith MA. *The Lancet* **2000**, *355*, 757.

[111] Benveniste H.; Einstein G.; Kim KR.; Hulette C.; Johnson GA. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14079.

[112] Carrell RW.; Lomas DA. *The Lancet.* **1997**, *350*, 134.

[113] Kaye JA. *Arch. Neurol.* **2000**, *57*, 312.

[114] Chustecka Z. *SCRIP* **1998**, *2325*, 22.

[115] SCRIP, **2000**, 2563, 22.

[116] Selkoe DJ. *JAMA* **2000**, *283*, 1615.

[117] Reixach N.; Crooks E.; Ostresh J.M.; Houghten R.A.; Blondelle S.E. *J. Struct. Biol.* **2000**, *130*, 247.

[118] Hideo Hirai T.; Toshio Ichiba K.; Hiroko Tonai T. *Patent application* **2001**, N° US 6,177,458.

[119] Kanfer J.N.; Sorrentino G.; Sitar D.S. *Neurochemical Res.* **1999**, *24*, 1621.

[120] Rajinder B.; Hai L.; Ratneshwar L. *FASEB J.* **2000**, *14*, 1233.

[121] Haugabook S.J.; Le T.; Yager D.; Zenk B.; Healy B.M.; Eckman E.A.; Prada C.; Younkin L.; Murphy P.; Pinnix I.; Onstead L.; Sambamurti T.E.; Golde D.; Dickson S.G.; Younkin S.G.; Eckman C.B. *FASEB J.* **2001**, *15*, 16.

[122] Hsiao K.; Chapman P.; Nilsen S.; Eckman C.; Harigaya Y.; Younkin S.; Yang F.; Cole G. *Science* **1996**, *274*, 99.